

Sexing Little Penguins *Eudyptula minor* using bill measurements

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Abstract

In Little Penguins *Eudyptula minor* there are no reliable plumage or body size differences that can be used visually to distinguish the sex of individuals. However, sexual dimorphism of morphometric measures has been noted, with males always being a little larger than females. In this study, differences between *E. minor* sexes at eight colonies in south-eastern Australia were determined statistically via discriminant function analysis (DFA) and through the utilization of DNA-based techniques developed for non-ratite birds. The DFA correctly determined gender in 91.1% of cases and molecular methods were 100% accurate. Our DFA success rate of classification is similar to that previously published for Little Penguins in Victoria. In this study statistically significant differences in mean bill depths and lengths were found between Little Penguin colonies at St Kilda, Phillip Island and Gabo Island, compared to colonies at Kangaroo Island, Granite Island, Middle Island and London Bridge. As birds in eastern populations (St Kilda, Phillip Island, Gabo Island) exhibit statistically significantly smaller beaks (bill depth and bill length), separate discriminant functions were investigated for each phenotypically distinct geo-spatial cohort. Interestingly, cluster analysis for bill length identified three groups; western (Kangaroo Island and Granite Island), eastern (St Kilda, Phillip Island and Middle Island) and the London Bridge Little Penguin colony, which constituted a separate group. We conclude that while there is a slight increase in DF power for colonies west of Cape Otway and for some specific colonies, colony-specific DFA is not required to identify the sex of Little Penguins in south-eastern Australia. (*The Victorian Naturalist* 123 (6) 2006, 390-395).

Introduction

The Little Penguin *Eudyptula minor* is the smallest penguin species and is endemic to temperate seas in Australia and New Zealand. Australia has one sub-species (*E. minor novaehollandiae*), found from Fremantle (WA) in the west to northern NSW and Tasmania in the southeast. There are five sub-species in New Zealand (Kinsky and Falla 1976). However, Banks *et al.* (2002) recently demonstrated that molecular results subdivide *E. minor* into only two clades: 1) the majority of New Zealand colonies and 2) Australia (sample from Phillip Island) and Otago *E. minor*.

In order to manage Little Penguin populations effectively, demographic analyses require the accurate determination of gender of the animals in the field (Caughley and Gunn 1996). However, Little Penguins show no differences in plumage between genders, and body size is also similar for males and females (Agnew and Kerry 1995).

Bill depth has been a useful sexually distinguishing morphological feature in Little Penguins from Tasmania (Gales 1988), New Zealand (Renner and Davis 1999, Hocken and Russell 2002) and also from

Phillip Island and Gibson Steps in Victoria (Arnould *et al.* 2004). Gales (1988) was the first to use a discriminant function (DF) that uses bill depth and length. The New Zealand work also developed DFs that demonstrated each sub-species required different functions. Arnould *et al.* (2004) found the DFs derived by workers for New Zealand populations of *E. minor* were poor predictors of gender for Little Penguins at Phillip Island and Gibson Steps. Gales' (1988) DF derived from a Tasmanian population yielded a reliability of 89.3% and 92.2% for birds at the two Victorian sites (Arnould *et al.* 2004) compared to 94% in Tasmania.

Preliminary results from our studies at seven colonies of Little Penguins in south-eastern Australia suggested bill dimensions of adults varied among colonies. Arnould *et al.* (2004) found similar differences in bill depth in their studies that prompted them to propose colony-specific DFs might be needed in order to determine the gender of birds accurately, rather than just using the one DF for the Australian sub-species.

The aim of this paper is to see whether it is possible to derive a single DF that can be used to determine accurately the gender of Little Penguins at eight sites in south-eastern Australia.

Methods

Bill depth (vertical thickness at the nostrils) and length (length at exposed culmen to tip of bill) were measured (± 0.1 mm) on 50 adult *E. minor* at seven of the sites shown in Fig. 1. To minimize inter-operator variation the same person took all measurements. Data provided in Arnould *et al.* (2004) for known-gender birds at Gibson Steps were used to test both Gales' (1988) and our overall discriminant function.

Blood samples were collected from the birds using standard techniques (Ellegren 1996) and gender determined genetically using the methods of Fridolfsson and Ellegren (1999) that rely on intron length variation in the sex chromosome-specific CHD (chromo-helicase-DNA binding protein) gene locus. This allowed us to know the gender of the birds that had previously had their bill measurements taken.

The molecular method of gender determination was verified by application to 40 Little Penguin carcasses from Middle Island, Warrnambool, that had been killed by foxes and subjected to gender determination by dissection.

Geographic variation in sexual dimorphism was tested using Kruskal-Wallis non parametric ANOVA. The Mann-Whitney test was used to assess for *post hoc* differences and a P-value of <0.05 was considered statistically significant. Discriminant function analysis was used to identify the gender of individual penguins. We used both bill depth (BD, mm) and bill length (BL, mm) in our DF. Assumptions associated with discriminant function analysis were not violated. The DF we derived was tested on 350 birds. Wilk's Lambda test was used to determine whether both variables (BD and BL) contributed significantly to the model. Canonical discriminant function coefficients were derived in order to establish the linear function (Gales 1988). Cluster analysis was undertaken with respect to location to determine if there were any distinct homogeneous sub sets.

Results

Examination of the seven sites for statistically significant differences (Table 1) in bill length as a function of location (Fig. 1) revealed that *E. minor* from the Kangaroo Island, Granite Island, Middle Island and London Bridge colonies have significantly longer bills when compared to birds from the more eastern colonies (Fig. 2a). Analysis of the seven sites for statistically significant differences in bill depth derived a similar result, with *E. minor* from Kangaroo Island, Granite Island and London Bridge having significantly deeper bills compared to *E. minor* from the more eastern colonies (Fig. 2b).

Cluster analysis was also performed on the variables Bill Length and Bill Depth as a function of location. Bill Length proved the more interesting variable with three groups identified: western (Kangaroo and Granite Island), eastern (St Kilda, Phillip Island and Middle Island) and the London Bridge Little Penguin colony, which is a separate group (Fig. 3). Gales' (1988) had $DF = -83.10 + (10.06 \ln BL) + (17.99 \ln BD)$, where DS is the discriminant score and \ln the natural logarithm. When we applied this DF, we found it produced differences in the success rate of classification for predicting the gender of Little Penguins (Table 1). The DF that Gales (1988) derived was most reliable for birds in the east of Victoria.

Arnould *et al.* (2004) derived the following DF for Little Penguins at Phillip Island and Gibson Steps: $DS = 1.242 BD - 16.774$. The DF model derived by Arnould *et al.* (2004) from Phillip Island and Gibson Steps *E. minor* colonies successfully determined sex for 88.3% of the *E. minor* observations from south-eastern Australia.

Testing each of the seven *E. minor* colonies separately for the DF model derived by Gales (1988) and Arnould *et al.* (2004) resulted in varying success. The accuracy of both DF models decreased at the Kangaroo Island, Granite Island, Middle Island and London Bridge colonies, while it increased at the St Kilda, Phillip Island and Gabo Island *E. minor* colonies (Table 2).

The DF we derived from all samples from all locations is: $DS = -18.710 + (1.292 BD) + (0.015 BL)$.

Table 1. Mann-Whitney *post hoc* one-tailed differences for bill length of *Eudyptula minor* from seven colonies tabulated (n = 50 individuals /colony, * statistically significant at the 0.05 level). Z = z-scores, P = p-value.

		Kangaroo Island	Granite Island	Middle Island	London Bridge	St Kilda	Phillip Island
Granite Island	Z	0.574		1.110	0.707	-3.149	3.623
	P	0.566		0.267	0.480	0.001*	0.000*
Middle Island	Z	-0.622	1.110		0.565	-1.968	2.385
	P	0.534	0.267		0.572	0.025*	0.009*
London Bridge	Z	-0.299	0.707	0.565		-2.907	3.167
	P	0.765	0.480	0.572		0.002*	0.001*
St Kilda	Z	-3.051	-3.149	-1.968	-2.907		0.128
	P	0.002*	0.002*	0.049*	0.004*		0.898
		K>StK	GrI>StK	MI>StK	LB>StK		
Phillip Island	Z	3.414	3.623	2.385	3.167	0.128	
	P	0.001*	0.000*	0.017*	0.002*	0.898	
		K>PI	GrI>PI	MI>PI	LB>PI		
Gabo Island	Z	-3.112	3.240	2.197	2.914	-0.073	0.130
	P	0.002*	0.001*	0.028*	0.004*	0.941	0.897
		K>GI	GrI>GI	MI>GI	LB>GI		

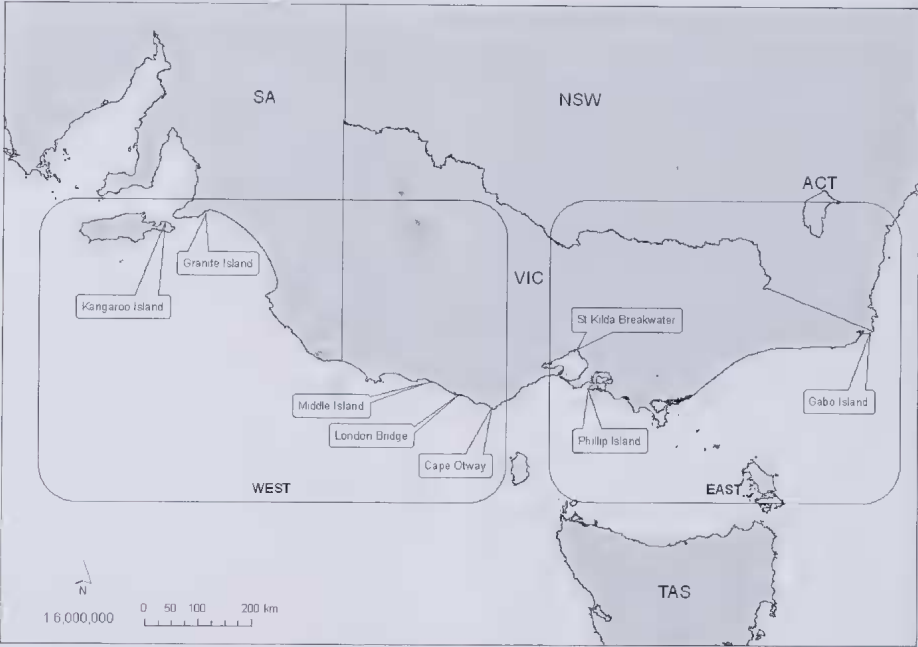


Fig. 1. The location of the eight colonies of Little Penguins used in this study.

Table 2. Geographically grouped and colony-specific DFs for the *Eudyptula minor* breeding in south-eastern Australia.

Location	Discriminant Function	Success rate of classification		
		This Study	Gales (1988) Tasmania DF	Arnould <i>et al.</i> (2004)
East of Cape Otway	= (BD X 1.425) + (BL X 0.048) - 21.312	91.3%		
West of Cape Otway	= (BD X 1.328) + (BL X 0.011) - 19.692	90.5%		
Kangaroo Island	= (BD X 1.443) + (BL X 0.042) -22.148	88.2%	73.0%	74.0%
Granite Island	= (BD X 1.362) + (BL X 0.107) -24.115	88.0%	80.0%	80.0%
Middle Island	= (BD X 1.452) + (BL X 0.049) -21.889	96.0%	76.0%	76.0%
London Bridge	= (BD X 1.37) + (BL X -0.038) -18.702	94.0%	76.0%	76.0%
St Kilda	= (BD X 1.236) + (BL X 0.139) -22.053	88.9%	88.9%	88.0%
Phillip Island	= (BD X 1.687) + (BL X 0.169) -29.578	94.3%	94.3%	93.0%
Gabo Island	= (BD X 1.341) + (BL X -0.039) -16.939	94.1%	84.3%	84.0%

Using all samples the DF correctly predicted gender in 91.1% of birds tested, with DS values >0 as male and those <0 females. This DF was almost as reliable as the one we derived for determining gender of birds in the eastern colonies (91.3%) compared with those in the west (90.5%). A discriminant function was developed for each of the seven sites, for sites clustered both east and west of Cape Otway, and overall, for all sites (Table 1).

Discussion

We found mean adult Little Penguin bill depth and length varied between the eight sites sampled in this study. This supports the observations reported by Arnould *et al.* (2004) who also found differences in bill depth in birds from different colonies; these differences prompted them to suggest there might be a need for a different DF for each colony in Australia. Fig. 2 indicates that birds from our sites clustered into two groups – one east of Cape Otway and one to the west. In all sites, the males had statistically significantly larger bills than the females.

When the DF derived by Gales (1988) in Tasmania was applied for the eight sites (the seven sites used in this study and Gibson Steps), gender was successfully determined in 81.7% of cases. When Gales’ DF was applied separately to the data from the seven colonies studied, the reliability was lowest for the western colonies and higher for the colonies at St Kilda, Phillip Island and Gabo Island.

Our DF correctly determined gender in birds from the eight colonies in 91.1% of

cases. Further, its reliability in determining gender of birds in the eastern colonies (91.3%) compared with those in the west (90.5%) was also high. The colony-specific DF success rates varied, with a high success of 96% for the Middle Island colony and a low of 88% for the Granite Island birds.

The DF we have derived is thus more reliable at predicting the gender of penguins in south-east Australia in comparison with other DFs that have been published for the same species elsewhere.

Acknowledgements

We thank several field assistants who assisted with data collection (N Overeem, A Overeem, T Murray, C McClusky, S Williamson, A Chiaradia and L Renwick) and colleagues at the Molecular Ecology and Biodiversity Laboratory at Deakin University. The project could not have been undertaken without the financial assistance of a Deakin University Post-Graduate Research Award and the Holsworth Wildlife Research Fund. The research was carried out with permission from the various governing bodies: Deakin University Animal Ethics Committee (permit number: A10/2003) the Department of Natural Resources and Environment/Sustainability and Environment approved fieldwork to be undertaken at Middle Island, London Bridge, St Kilda and Gabo Island (permit number: 10002229) while in South Australia the Department of Environment approved work at Kangaroo and Granite Islands (permit number: 10/2003) and the Department of Environment and Heritage (Z24663). In addition, the Warrnambool City Council (through the Coast and Rivers Advisory Committee) and the St Kilda Penguin Study Group approved fieldwork at Middle Island and St Kilda.

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Received 20 October 2005; accepted 16 November 2006

Is there always a bias towards young males in road kill samples? The case in Victorian Koalas *Phascolarctos cinereus*

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Abstract

Mortality due to road trauma can have large negative impacts on some populations and often is biased towards age/sex classes that have higher rates of movement; individuals during the breeding season and juveniles while they are dispersing. A bias towards young males has been found in two previous studies of road kill Koalas in southeast Queensland. Such a bias was not found in the present study of Koala skulls from populations across Victoria. This may be due to the different Koala population structures and densities or road types and characteristics. (*The Victorian Naturalist* **123** (6) 2006, 395-399).

Introduction

Individuals of many species are killed on the roads (Trombulak and Frissell 2000; Taylor and Goldingay 2004) and this can have large negative effects on populations of wild animals (Dufty 1994; Jones 2000; Hebblewhite *et al.* 2003; Lopez *et al.* 2003). Road trauma is known to impact some Koala populations (Backhouse and Crouch 1990; Lunney *et al.* 1996; Thompson 1996). The Phillip Island Koala population in Victoria decreased substantially between 1973 and 1988, mostly due to high mortality from road trauma (Every 1986; Backhouse and Crouch 1990).

Mortality rates due to road trauma have been found to differ between temporal seasons (Taylor and Goldingay 2004) and may be greater in age/sex classes that have

high dispersal rates or increased activity levels (Bonnet *et al.* 1999, Inbar and Mayer 1999). For example, Coulson (1989) found that 48% of road killed Eastern Grey Kangaroos *Macropus giganteus* were 1 to 2 years of age, the age when they were dispersing. A significant bias towards road kills of two-year-old macropods was also found by Lee *et al.* (2004). Additionally, males were more likely to be hit than females in five species of macropods, possibly because of their greater ranging behaviour (Coulson 1997). A high proportion of ungulate road traumas are also related to dispersal and breeding behaviour (Groot Bruinderink and Hazebroek 1996). Similar patterns of male-biased mortality caused by road trauma have been found in